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Immediate GTP hydrolysis upon FtsZ polymerization

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Summary

To understand the polymerization dynamics of FtsZ, a bacterial cell division protein similar to tubulin, insight is required into the nature of the nucleotide bound to the polymerized protein. In a previous study, we showed that the FtsZ polymers contain mostly GDP. A recent study challenged this result, suggesting that the polymerized FtsZ is in a GTP-bound state. Here, we show that, when radiolabelled [γ-^{32}P]-GTP is used to polymerize FtsZ, GTP is hydrolysed instantaneously. The FtsZ polymer contains both GDP and the radiolabelled inorganic phosphate.

Introduction

FtsZ is a key prokaryotic cell division protein, forming a structural element known as the Z-ring at the site of cell division (reviewed by Margolin, 2000; Scheffers and Driessen, 2001). In *Escherichia coli*, the Z-ring is critical for the localization of all other protein components of the cell division machinery. The Z-ring is likely to consist of polymers similar to the FtsZ polymers that can be formed *in vitro* in a GTP-dependent manner (Bramhill and Thompson, 1994; Erickson *et al*., 1996; Mukherjee and Lutkenhaus, 1998). FtsZ is a 40.3 kDa protein that binds and hydrolyses GTP (De Boer *et al*., 1992; RayChaudhuri and Park, 1992; Mukherjee *et al*., 1993). It shows a striking structural similarity to α- and β-tubulins, both as a monomer and when modelled onto protofilaments (Löwe and Amos, 1998; 1999; Nogales *et al*., 1998; 1999). FtsZ and tubulins share a unique GTP-binding motif, the G-box [GGGTG(ST)G] (De Boer *et al*., 1992).

Structural modelling of the FtsZ crystal structure onto electron microscopy images of the FtsZ protofilament strongly indicated that the active site for GTP hydrolysis is shared between two FtsZ subunits. In this organization, the GTP-binding pocket is provided by one subunit, while the GTPase-activating T7 loop that could be involved in Mg^{2+} co-ordination comes from the other subunit (Löwe and Amos, 1999). This structural organization is similar to that of the α,β-tubulin heterodimer in microtubules (Nogales *et al*., 1999). Work in our laboratory provided biochemical evidence in favour of this model: first, a critical aspartate residue in the T7 loop is involved in cation co-ordination (Scheffers *et al*., 2001) and, secondly, several T7 loop mutants inhibited in GTP hydrolysis suppress the GTPase activity of wild-type FtsZ when mixed (Scheffers *et al*., 2002).

With the active site for GTP hydrolysis formed upon association of FtsZ monomers, it is likely that GTP hydrolysis closely follows FtsZ polymerization. Various reports have shown that GTPase activity of FtsZ occurs only at FtsZ concentrations that allow the formation of large FtsZ polymers (Wang and Lutkenhaus, 1993; Lu *et al*., 1998; Mukherjee and Lutkenhaus, 1998; Sossong *et al*., 1999). This is indicative of self-activation of the FtsZ GTPase activity: association of FtsZ monomers triggers the GTPase activity of FtsZ. However, several other reports describe the non-co-operative formation of FtsZ protofilaments (Rivas *et al*., 2000; 2001; Romberg *et al*., 2001) with GTP hydrolysis predicted to lag behind polymerization.

One of the major questions in the study of FtsZ polymerization is the state of the nucleotide bound to the FtsZ polymer. Previously, we reported that the FtsZ polymer predominantly contains GDP (Scheffers *et al*., 2000). However, Mingorance *et al*. (2001) have recently presented results from which it was concluded that FtsZ polymers formed in solution mostly contain GTP. Combined with nucleotide exchange experiments, it was concluded that, during maximal FtsZ polymerization, GDP is not a significant fraction of the bound nucleotide and that nucleotide exchange can occur on all polymer subunits, irrespective of the nature of the bound nucleotide. These conclusions contradict our previous findings. Here, we provide further evidence to show that the FtsZ polymer contains GDP and that the inorganic phosphate formed upon the hydrolysis of GTP is retained by the polymer.

Results

Nucleotide content of FtsZ at limiting GTP concentrations

Briefly, Mingorance *et al*. (2001) described experiments in

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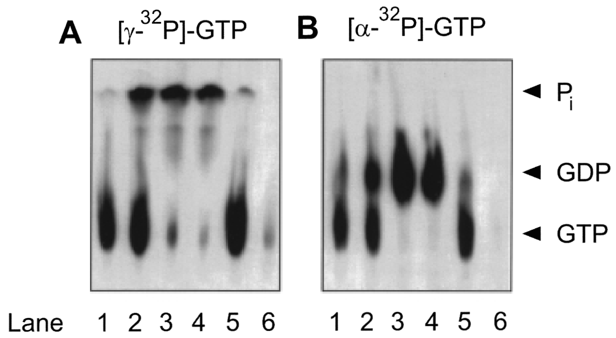

Fig. 1. Analysis of FtsZ-bound nucleotides by precipitation and thin-layer chromatography. FtsZ (0.5 mg ml^{-1}), equilibrated in 50 mM Mes/NaOH, pH 6.5, 50 mM KCl with additions as described below, was incubated in the same buffer at 30°C, and either [γ-^{32}P]-GTP (A) or [α-^{32}P]-GTP (B) was added to 20 μM. Immediately after the addition of GTP, FtsZ was precipitated using ammonium sulphate, and the nucleotides were extracted using ice-cold perchloric acid and analysed by TLC (Scheffers *et al*., 2000). Lane 1, no additions; lane 2, 10 mM $CaCl_2$; lane 3, 5 mM $MgCl_2$; lane 4, 5 mM $MgCl_2$, 10 mM $CaCl_2$; lane 5, 1 mM EDTA, 1 mM EGTA; lane 6, control: BSA (0.5 mg ml^{-1}) in 50 mM Mes/NaOH, pH 6.5, 50 mM KCl, 5 mM $MgCl_2$.

which radiolabelled GTP was added to FtsZ, after which the amount of FtsZ-bound nucleotide was determined by separation of the protein-bound nucleotide from free nucleotide by binding of the protein to nitrocellulose filters. [α-^{32}P]-GTP was used to determine the total nucleotide (GTP+GDP) content of the protein, whereas [γ-^{32}P]-GTP was used to determine the amount of GTP bound to the protein. As retention of radioactivity with [γ-^{32}P]-GTP is represented as GTP bound to FtsZ, the interpretation of the experiment is based on the assumption that, after GTP hydrolysis, the release of inorganic phosphate is very rapid. The presence of a small fraction of the protein containing bound GDP+P_i was not ruled out but, without quantitative data, it is questionable as to whether this really only concerns a small fraction.

We have carried out similar experiments, but used thin-layer chromatography (TLC) to identify the label bound to protein. For this purpose, FtsZ was incubated with different radiolabelled nucleotides under specified conditions, and the protein was quickly recovered from the solution by ammonium sulphate precipitation. Nucleotides bound to the protein fraction were extracted using perchloric acid and analysed by TLC. We used limiting concentrations of GTP to prevent significant exchange of fresh nucleotide, which would make the results more difficult to interpret (see below). The amount of GTP used is sufficient to allow the formation of polymers in the presence of 5 mM Mg^{2+} and 10 mM Ca^{2+}, as was shown previously by both light scattering and electron microscopy (Scheffers *et al*., 2000). FtsZ that is incubated in cation-containing buffers (Mg^{2+}, Ca^{2+} or both) immediately hydrolyses GTP upon addition of [α-^{32}P]-GTP or [γ-^{32}P]-GTP (Fig. 1). When Ca^{2+} is the only cation present, a significant portion of the GTP is not hydrolysed immediately and recovered as [γ-^{32}P]-GTP bound to FtsZ (Fig. 1A, lane 2). However, as soon as Mg^{2+} is included, practically all the FtsZ-bound nucleotide is GDP (Fig. 1B, lanes 3 and 4). When [γ-^{32}P]-GTP is used, hydrolysed $^{32}P_i$ co-precipitates with the protein fraction indicating that it remains bound to FtsZ (Fig. 1A, lanes 3 and 4). This demonstrates that the radioactivity retained by filtration of the FtsZ incubated with [γ-^{32}P]-GTP observed by Mingorance *et al*. (2001) must represent radiolabelled inorganic phosphate. As radiolabelled inorganic phosphate is also retained after hydrolysis in the absence of Ca^{2+} (Fig. 1A, lane3), we exclude the possibility that the spots are the result of $Ca_3(PO4)_2$ precipitation. Moreover, the perchloric acid extraction of the bound nucleotide did not induce the hydrolysis of GTP, as GTP-bound FtsZ could be recovered when the protein was incubated with GTP in the presence of the chelators EDTA and EGTA to prevent hydrolysis (Fig. 1A and B, lane 5). When FtsZ was replaced by an unrelated protein, i.e. bovine serum albumin (BSA), no nucleotide could be recovered in the pellet fraction after ammonium sulphate precipitation (Fig. 1A and B, lane 6).

Nucleotide content of FtsZ at saturating GTP concentrations

To ensure that the observed retention of inorganic phosphate is also valid at higher GTP concentrations that lead to significant polymerization in the presence of cations (Mukherjee and Lutkenhaus, 1999), the experiments were repeated with 1 mM GTP. Moreover, to validate the ammonium sulphate precipitation assay, we used both precipitation (Scheffers *et al*., 2000) and filtration (Mingorance *et al*., 2001) to analyse the nucleotide contents of the polymers. For this purpose, the nucleotide retained on the filter was immediately extracted with ice-cold perchloric acid and analysed by TLC. Even when polymer mixtures were filtered immediately after the addition of label, a significant GTP hydrolysis and retention of radiolabelled inorganic phosphate was observed (Fig. 2). Identical results were obtained when the FtsZ was recovered by ammonium sulphate precipitation (not shown). These data also demonstrate that, at high GTP concentrations, the FtsZ polymer mostly contains bound GDP.

Discussion

In the description of models for FtsZ polymerization, the kinetics of nucleotide hydrolysis, release and/or exchange play a pivotal role. Previously, we have shown that FtsZ polymers containing mostly GDP can be stabilized by GTP-γ-S (Scheffers *et al*., 2000), suggesting that FtsZ

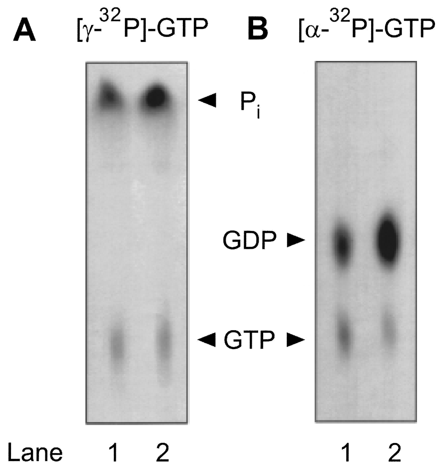

Fig. 2. Analysis of FtsZ-bound nucleotides by filtration and TLC. FtsZ (0.5 mg ml^{-1}), was incubated at room temperature in 50 mM Mes/NaOH (pH 6.5), 50 mM KCl with 5 mM $MgCl_2$ (lane 1) or 5 mM $MgCl_2$, 10 mM $CaCl_2$ (lane 2). Either [γ-^{32}P]-GTP (A) or [α-^{32}P]-GTP (B) was added to 1 mM. Immediately after the addition of GTP, FtsZ was recovered on nitrocellulose membrane filters. Subsequently, nucleotides bound to FtsZ were extracted using ice-cold perchloric acid in the presence of EDTA and analysed by TLC.

polymers are stabilized by a GTP cap, similar to tubulin (reviewed by Desai and Mitchison, 1997). In other reports, it has been suggested that FtsZ polymers contain mostly GTP. This was based on either (theoretical) modelling of the polymerization reaction (Romberg *et al*., 2001) or recovery of radiolabelled nucleotide with FtsZ using a filtration assay (Mingorance *et al*., 2001). Here, we present a direct identification of the nucleotide content of FtsZ under polymerizing conditions. The data show that GDP is always a major fraction of the FtsZ-bound nucleotide fraction, whereas the inorganic phosphate formed after hydrolysis is retained by the polymer. The filtration assay used by Mingorance *et al*. (2001) does not discriminate between [γ-^{32}P]-GTP bound to FtsZ or GDP plus $^{32}P_i$. Our findings show that those experiments that were interpreted as FtsZ polymers containing mostly GTP, in fact reflect FtsZ polymers containing mostly GDP with retention of the inorganic phosphate after GTP hydrolysis.

Mingorance *et al*. (2001) noticed that [γ-^{32}P]-GTP associated with the FtsZ polymer (Fig. 3B in their paper) was only slowly released concomitantly with depolymerization. We interpret their experiment as retention of $^{32}P_i$ during the first minutes of polymerization, followed by a slow release at longer times. However, after complete depolymerization, about 50% of the radiolabel is still bound to the protein. We have also observed retention of labelled P_i and GDP after complete depolymerization (not shown; Scheffers *et al*., 2000).

The immediate hydrolysis of GTP when added to FtsZ in the presence of cations has important implications for the FtsZ polymerization models described in the literature. The observed rapid exchange of bound radiolabelled nucleotides on the polymer with added GTP (Mingorance *et al*., 2001) does not reflect the exchange of FtsZ-bound GTP, but FtsZ-bound GDP with GTP from solution. Further, the model for isodesmic FtsZ polymerization, in which most of the FtsZ subunits in the polymer contain GTP (Romberg *et al*., 2001), seems unlikely given our biochemical data. The immediate GTP hydrolysis we describe here fits a model in which the active site for GTP hydrolysis is formed by the contact between two FtsZ monomers, leading to immediate hydrolysis of the bound GTP upon FtsZ–FtsZ association (reviewed by Scheffers and Driessen, 2001). Previously, we have presented evidence pointing out that the stability of FtsZ polymers may be defined by the presence of a 'GTP cap' as described for tubulin (Scheffers *et al*., 2000). It remains to be determined whether this cap consists of GTP-bound FtsZ or GDP-P_i-bound FtsZ. The organization of microtubules suggests that the microtubule plus end is always capped by at least one GTP-bound tubulin heterodimer. Whether microtubules are stabilized by GDP-P_i is, however, still a matter of debate (reviewed by Nogales, 2000). Further polymer stability could be provided by the rapid exchange of GDP for GTP within the polymer (Mingorance *et al*., 2001). More evidence for co-operative assembly of FtsZ polymers and stabilization of the FtsZ polymer by a GTP cap was described recently by Mukherjee *et al*. (2001).

In this report, we show that GTP is hydrolysed immediately by FtsZ when added to FtsZ at polymerizing conditions, with retention of P_i. The role of P_i release in the polymerization of FtsZ has hardly been addressed to date, with most reports in the literature solely considering GTP- or GDP-bound FtsZ. Recently, it has been shown that the γ-phosphate of GTP in FtsZ is sensed by the T3 loop, similar to tubulin (Díaz *et al*., 2001). Molecular dynamic studies using the FtsZ crystal structure showed that GTP-bound FtsZ has a more compact conformation than GDP-bound FtsZ, and P_i release may have a decisive impact on polymer conformation and stability (Díaz *et al*., 2001). We have obtained evidence that a conserved positive charge, located in the T7 loop region of FtsZ, may also be implicated in co-ordination of the binding and release of P_i after GTP hydrolysis (Scheffers *et al*., 2002). Combined with the data in this report, further studies on the dynamics of P_i retention and release from the FtsZ polymer are required.

Experimental procedures

Materials

FtsZ was purified as described previously (Scheffers *et al*., 2000), and the FtsZ concentration was determined using a Bradford assay with a correction factor of 0.82 for the FtsZ:BSA ratio (Lu *et al*., 1998). [α-^{32}P]-GTP (3000 Ci mmol^{-1})

and [γ-^{32}P]-GTP (5000 Ci mmol^{-1}) were obtained from Amersham Pharmacia Biotech. Other chemicals were obtained from Sigma-Aldrich.

Analysis of FtsZ-bound nucleotides by TLC

FtsZ (0.5 mg ml^{-1}) was incubated at 30°C in 50 mM Mes/NaOH (pH 6.5), 50 mM KCl (buffer A) and cations when indicated. Either [γ-^{32}P]-GTP or [α-^{32}P]-GTP was added to the concentrations indicated, and the FtsZ was recovered by filtration or ammonium sulphate precipitation. In the case of the filtration assay, the FtsZ–GTP mixtures (20 μl) were filtered through nitrocellulose disks (pore size 45 μm; Whatman) and washed with 400 μl of wash buffer [20 mM Tris-HCl, pH 8.0, 50 mM NaCl, 5 mM $MgSO_4$, 0.005% Triton X-100, 20 mM $(NH4)_2SO_4$] as described previously (Mingorance *et al*., 2001). Subsequently, the filters were extracted using 50 μl of ice-cold perchloric acid solution (a 1:2 v/v mixture of 14% perchloric acid, 9 mM EDTA and buffer A). The pH of the samples was adjusted using 25 μl of 1 M $KHCO_3$, 1 M KOH, and the samples were clarified by centrifugation for 3 min at 20 000 *g*. Samples of 10 μl were spotted on CEL 300 PEI/UV_{254} plates (Macherey Nagel) and analysed by TLC using 0.65 M KH_2PO_4/H_3PO_4, pH 3.5, as running buffer. Plates were dried and exposed to Kodak Biomax MR films. For the precipitation assay, the FtsZ–GTP mixture (20 μl) was precipitated using 20 μl of a 75% (w/v) ammonium sulphate solution in buffer A immediately upon addition of GTP (handling time between the addition of GTP and precipitation was <10 s). Precipitation was performed on ice for 10 min, and the protein was recovered by centrifugation for 5 min at 20 000 *g*. Subsequently, nucleotides bound to FtsZ were extracted using perchloric acid and analysed by TLC as described above.

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References

Bramhill, D., and Thompson, C.M. (1994) GTP-dependent polymerization of *Escherichia coli* FtsZ protein to form tubules. *Proc Natl Acad Sci USA* **91:** 5813–5817.

De Boer, P.A.J., Crossley, R.E., and Rothfield, L.I. (1992) The essential bacterial cell-division protein FtsZ is a GTPase. *Nature* **359:** 254–256.

Desai, A., and Mitchison, T.J. (1997) Microtubule polymerization dynamics. *Annu Rev Cell Dev Biol* **13:** 83–117.

Díaz, J.F., Kralicek, A., Mingorance, J., Palacios, J.P., Vicente, M., and Andreu, J.M. (2001) Activation of cell division protein FtsZ: control of switch loop T3 conformation by the nucleotide γ-phosphate. *J Biol Chem* **276:** 17307–17315.

Erickson, H.P., Taylor, D.W., Taylor, K.A., and Bramhill, D. (1996) Bacterial cell division protein FtsZ assembles into protofilament sheets and minirings, structural homologs of tubulin polymers. *Proc Natl Acad Sci USA* **93:** 519–523.

Löwe, J., and Amos, L.A. (1998) Crystal structure of the bacterial cell-division protein FtsZ. *Nature* **391:** 203–206.

Löwe, J., and Amos, L.A. (1999) Tubulin-like protofilaments in Ca^{2+}-induced FtsZ sheets. *EMBO J* **18:** 2364–2371.

Lu, C., Stricker, J., and Erickson, H.P. (1998) FtsZ from *Escherichia coli*, *Azotobacter vinelandii*, and *Thermotoga maritime* – quantitation, GTP hydrolysis, and assembly. *Cell Motil Cytoskeleton* **40:** 71–86.

Margolin, W. (2000) Themes and variations in prokaryotic cell division. *FEMS Microbiol Rev* **24:** 531–548.

Mingorance, J., Rueda, S., Gómez-Puertas, P., Valencia, A., and Vicente, M. (2001) *Escherichia coli* FtsZ polymers contain mostly GTP and have a high nucleotide turnover. *Mol Microbiol* **41:** 83–91.

Mukherjee, A., and Lutkenhaus, J. (1998) Dynamic assembly of FtsZ regulated by GTP hydrolysis. *EMBO J* **17:** 462–469.

Mukherjee, A., and Lutkenhaus, J. (1999) Analysis of FtsZ assembly by light scattering and determination of the role of divalent metal cations. *J Bacteriol* **181:** 823–832.

Mukherjee, A., Dai, K., and Lutkenhaus, J. (1993) *Escherichia coli* cell division protein FtsZ is a guanine nucleotide binding protein. *Proc Natl Acad Sci USA* **90:** 1053–1057.

Mukherjee, A., Saez, C., and Lutkenhaus, J. (2001) Assembly of an FtsZ mutant deficient in GTPase activity has implications for FtsZ assembly and the role of the Z ring in cell division. *J Bacteriol* **183:** 7190–7197.

Nogales, E. (2000) Structural insights into microtubule function. *Annu Rev Biochem* **69:** 277–302.

Nogales, E., Downing, K.H., Amos, L.A., and Löwe, J. (1998) Tubulin and FtsZ form a distinct family of GTPases. *Nature Struct Biol* **5:** 451–458.

Nogales, E., Whittaker, M., Milligan, R.A., and Downing, K.H. (1999) High-resolution model of the microtubule. *Cell* **96:** 79–88.

RayChaudhuri, D., and Park, J.T. (1992) *Escherichia coli* cell-division gene ftsZ encodes a novel GTP-binding protein. *Nature* **359:** 251–254.

Rivas, G., Lopez, A., Mingorance, J., Ferrandiz, M.J., Zorrilla, S., Minton, A.P., *et al.* (2000) Magnesium-induced linear self association of the FtsZ bacterial cell division protein monomer. The primary steps for FtsZ assembly. *J Biol Chem* **275:** 11740–11749.

Rivas, G., Fernandez, J.A., and Minton, A.P. (2001) Direct observation of the enhancement of noncooperative protein self-assembly by macromolecular crowding: indefinite linear self association of bacterial cell division protein FtsZ. *Proc Natl Acad Sci USA* **98:** 3150–3155.

Romberg, L., Simon, M., and Erickson, H.P. (2001) Polymerization of FtsZ, a bacterial homolog of tubulin: Is assembly cooperative? *J Biol Chem* **276:** 11743–11753.

Scheffers, D.J., and Driessen, A.J.M. (2001) The polymerization mechanism of the bacterial cell division protein FtsZ. *FEBS Lett* **506:** 6–10.

Scheffers, D.J., den Blaauwen, T., and Driessen, A.J.M. (2000) Non-hydrolysable GTP-γ-S stabilizes the FtsZ polymer in a GDP-bound state. *Mol Microbiol* **35:** 1211–1219.

Scheffers, D.J., de Wit, J.G., den Blaauwen, T., and Driessen, A.J.M. (2001) Substitution of a conserved aspartate allows cation-induced polymerization of FtsZ. *FEBS Lett* **494:** 34–37.

Scheffers, D.J., de Wit, J.G., den Blaauwen, T., and Driessen, A.J.M. (2002) GTP-hydrolysis of cell division protein FtsZ: evidence that the active site is formed by the association of monomers. *Biochemistry* **41:** 521–529.

Sossong, T.M., Jr, Brigham-Burke, M.R., Hensley, P., and Pearce, K.H., Jr (1999) Self-activation of guanosine triphosphatase activity by oligomerization of the bacterial cell division protein FtsZ. *Biochemistry* **38:** 14843–14850.

Wang, X., and Lutkenhaus, J. (1993) The FtsZ protein of *Bacillus subtilis* is localized at the division site and has GTPase activity that is dependent upon FtsZ concentration. *Mol Microbiol* **9:** 435–442.

